IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2663
Steven M. RUBEN	Art Unit: 1644
Appl. No.: 10/662,429	Examiner: HUYNH, PHUONG N.
Filed: September 16, 2003	Atty. Docket: 1488.1890003/EJH/SAC
For: **Apoptosis Inducing Molecule I**	

Declaration of Steven M. Ruben
Ruben Exhibit #157

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Declaration of Steven M. Ruben
Ruben Exhibit #157

Paper No. _____

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES
(Administrative Patent Judge Sally Gardner Lane)

STEVEN M. RUBEN

Junior Party,
(Application No. 08/816,981),

v.

STEVEN R. WILEY
and RAYMOND G. GOODWIN

Senior Party,
(Patent No. 5,763,223).

Patent Interference No. 105,077

DECLARATION OF STEVEN M. RUBEN

Ruben EXHIBIT 2157
Ruben v. Wiley et al.
Interference 105,077

Paper No. _____

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Wiley EXHIBIT 1032
Ruben v. Wiley et al
Interference 105,077

Paper No. _____

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DECLARATION OF STEVEN M. RUBEN

Ruben EXHIBIT 2102
Ruben v. Wiley et al.
Interference No. 105,077
RX 2102

DECLARATION OF STEVEN M. RUBEN

I, STEVEN M. RUBEN, declare and state as follows:

1. I am the same Steven M. Ruben named as the inventor for involved United States patent application 08/816,981. I joined Human Genome Sciences, Inc. (HGS) in 1992 in the position of Scientist, a position I held until 1993. Then, I served as Associate Director of the Molecular Biology Department of HGS from 1993 to 1996, and as Director of the Molecular Biology Department from 1996 to 1998. In 1999, I became Vice President, Research, and eventually became Vice President of Preclinical Discovery, the position that I held when I left HGS in March 2003. I am currently Vice President of Protein Therapeutics at Celera.

2. As Associate Director of the Molecular Biology Department of HGS in the 1993 to 1996 time frame, I supervised and directed the development of a novel Fas Ligand homolog that came to be known as AIM-I (Apoptosis Inducing Molecule-I), an HGS high priority therapeutic protein candidate. During this time, I was responsible for seven Ph.D. scientists and support staff who were focussing on therapeutic protein identification and biological characterization of candidate genes, including AIM-I.

3. AIM-I is referred to by various synonyms in this showing and the supporting documents. In the Wiley '223 patent, AIM-I is referred to as TRAIL, for *T*NF-related *a*poptosis *i*nducing *l*igand. As explained below, the HGS clone encoding AIM-I is termed HTPAN08. Other identifiers for sequences encoding AIM-I include HTPAN08SO4, 413412 (HGS code) and ATG 343 (code of HGS's collaborators at SmithKline Beecham (see ¶ 32 below)). The AIM-I protein itself is alternatively referred to as TL2, TL-2, TNFL1, Apo2 ligand, APO-2L, and as a homolog of Fas ligand from rat ("rat Fas ligand homolog" or "Fas Ligand" or "FasLig").

4. One of the scientific staff members whom I supervised was Ann Ferrie (formerly known as Ann M. Kim). From 1993 through March 14, 1996, Ms. Ferrie was the primary

individual in my laboratory responsible for carrying out, under my supervision, the benchwork experiments to develop AIM-I as a therapeutic candidate. The AIM-I project was continuously a priority project for Ms. Ferrie throughout this time period. Ms. Ferrie and I would discuss results of her work, including her AIM-I work, on a regular basis, generally two to three times per week, and together we would review the data in her notebook regularly, at which time I would direct the course of the experiments she was to carry out. I would also periodically independently review Ms. Ferrie's laboratory notebooks. I have recently reviewed nine of Ms. Ferrie's Notebooks (HGS Notebook Numbers 82, 127, 168, 202, 236, 270, 345, 405, and 489, also labeled by Ms. Ferrie as Ann Kim #3, #5, #6, #7, #8, #9, #10, #11, and #12; RE87 - RE95 respectively), as well as a loose leaf three ring binder that she maintained, containing sequencing orders and analyses such as BLAST analyses and sequence alignments that Ms. Ferrie performed under my direction specifically for AIM-I (HTPAN08) RE96). As an advanced Research Associate, Ms. Ferrie could follow through on her discussions with me to successfully complete ongoing projects. At the beginning of the AIM-I project, in the 1993 to 1994 time frame, our meetings occurred nearly every day and I would directly instruct her in such tasks as performing BLAST analyses and sequence alignments. As Ms. Ferrie became more experienced, I continued to supervise and direct her work, but she became more independent in carrying out her everyday bench experiments. Ms. Ferrie worked on developing reagents and characterizing AIM-I so the biological activity of the protein could be pursued further in HGS's interdepartmental program for this therapeutic candidate.

5. Each individual bench researcher at HGS, including Ms. Ferrie, maintained a laboratory notebook record of their daily experimental activities. In addition, HGS maintained a separate computerized database known during the 1994-1996 time frame as "IRIS". The IRIS

database summarized company projects on a Project Worksheet form that was an integral part of the technical information record-keeping process at HGS. The content of a Project Worksheet form entered and maintained in the IRIS database was updated by the responsible HGS staff scientists as he or she deemed necessary. The entire content of the electronic database during the time since at least the inception of the AIM-I project was periodically recorded on tape and archived (RE56).

6. On February 2, 1994, I created an electronic project for HTPAN08 (RE97 , subsequently revising it on April 14, 1995 (RE99). Specifically, the entry under "POTENTIAL COMMERCIAL VALUE" on the Project Worksheet created on February 2, 1994 (RE97) indicates that clone HTPAN08 encodes " . . . a potential cytokine with some homology to TNF alpha. TNF alpha has cytotoxic properties against several tumor lines." RE97 at page 1). In addition, Ms. Ferrie created an electronic project for HTPAN08 on February 8, 1994 RE98 . The BLAST results of the HTPAN08-encoded protein shown in that project worksheet indicate a high degree of homology with rat FasL, TNFα, and lymphotoxin beta, members of the TNF ligand family (RE98).

7. The BLAST search results recorded in the HGS electronic database in February, 1994, clearly indicated that AIM-I had the greatest similarity to Fas ligand, a TNF ligand family member known to induce apoptosis. Accordingly, I expected that AIM-I would also have apoptosis inducing activity. The BLAST analysis indicated that AIM-I was also similar to other TNF ligand family members. Indeed, after rat Fas ligand, the next nearest "hits" in the BLAST search results were all to various forms of TNFα, another TNF ligand family member that was known to induce apoptosis. RE98).

8. The degree of sequence similarity at key amino acid residues in the carboxy terminal extracellular region of the AIM-I protein with Fas Ligand and TNFα indicated to me in February 1994 that AIM-I would trigger apoptosis. Apoptosis, or programmed cell death, is a specific cellular self-termination protocol which is distinct from necrotic cell death and is characterized by DNA fragmentation or "laddering" and other hallmarks. This protocol is triggered by a subset of ligands within the TNF ligand family. Prototypic examples of such ligands known to induce apoptosis are Fas ligand, TNFα, and LTα. Abundant evidence existed at least as early as 1994 to inform scientists of the well-defined nature of the TNF ligand family (see, *e.g.*, Smith et al., 1994, Cell 76:959-962 (RE03). One of the reasons the TNF ligand family is so well established is because TNFα and LTα (a.k.a. lymphotoxin or TNFβ) were among the very first cytokines to be molecularly cloned and sequenced in 1984 and, by 1994, it was concluded that "[t]he contingent ability to induce death is rather unique to [the TNF ligand] family and is well established for TNFα, LTα, and FasL" (Smith (RE03) at page 962, column 1).

9. Additional evidence existed at least as early as 1994 demonstrating that the TNF ligand family contained members, such as Fas Ligand and TNFα, capable of triggering apoptosis (see, *e.g.*, Sachs and Lotem, 1993, Blood 82:15-21 RE107 ; Suda et al., 1993, Cell 75:1169-1178 RE108; Cosman, 1994, Stem Cells 12:440-455 RE109; Nagata, 1994, Phil. Trans. R. Soc. Lond., Series B: Biol. Sci. (England) 345:281-287 RE110; Nagata, 1994, Adv. Immunol. 57:129-144 RE111; Schulze-Osthoff, 1994, Trends Cell Biol. 4:421-426 RE112 Singer et al., 1994, Curr. Opin. Immunol. (England) 6:913-920 (RE113; Nagata, 1994, Semin. Immunol. 6:3-8 RE114; and Nagata et al., 1995, Science 267:1449-1456 RE115

The foregoing publications reflect my general knowledge in early 1995 regarding the ability of TNF ligand family members to induce apoptosis.

10. Moreover, considerable insight had been achieved by the time I identified AIM-I as a member of the TNF family into the specific functioning of certain ligand-receptor pairs within the TNF ligand and TNF receptor families, as evidenced by Watanabe-Fukunaga, 1992, Nature 356:314-317 RE116 Cohen et al., 1992, Immunol. Today 13:427-428 RE117; Crispe, 1994, Immunity 1:347-349 RE118 ; Lynch et al., 1994, Immunity 1:131-136 RE119 Takahashi et al., 1994, Cell 76:969-976 RE120; and Nagata et al., 1995, Immunol. Today (England) 16:39-43 RE121. The foregoing publications reflect my general knowledge in early 1995 regarding the associations between TNF ligand and TNF receptor family members.

11. From numerous experiments performed worldwide by a large number of laboratories in recent years, AIM-I has become quite well established as having apoptosis-inducing activity. Indeed, AIM-I and derivatives and fragments of AIM-I are considered leading candidates for therapeutic use in a number of proliferative disease settings. Eight review articles regarding AIM-I provide evidence of the foregoing and discuss the background of AIM-I (Nguyen et al., 2000, Forum (Genova) 10:243-52 RE106 ; Schneider et al., 2000, Pharm. Acta Helv. 74:281-6 RE122; Walczak et al., 2000, Exp. Cell Res. 256:58-66 RE123; Roth et al., 1999, Cell. Mol. Life Sci. 56:481-506 RE124; Marsters et al., 1999, Recent Prog. Horm. Res. 54:225-34 RE125; Bonavida et al., 1999, Int. J. Oncol. 15:793-802 RE126; Griffith et al., 1998, Curr. Opin. Immunol. 10:559-63 RE127; and Golstein, 1997, Curr. Biol. 7:R750-3 RE128.

SEQUENCE AND CHARACTERIZATION OF HTPAN08

12. On August 8, 1994, Ms. Ferrie printed out the nucleotide sequence and corresponding amino acid sequence of AIM-I (RE96 at pages 73-78). These sequences correspond to the AIM-I sequences disclosed in Figure 1 of Provisional Application No. 60/013,405, filed March 14, 1996 naming me as the inventor. (RE52 , Figure 1).

13. On August 9, 1994, under my instruction, Ms. Ferrie performed a BLAST analysis of the amino acid sequence of AIM-I, which revealed high degrees of similarity between AIM-I and several species of TNF alpha and Fas ligand proteins (RE96 at pages 71-72 and 81-85).

14. On August 10, 1994, under my direction, Ms. Ferrie aligned the AIM-I protein amino acid sequence with several other members of the TNF ligand family to highlight the regions of homology among the proteins, including TNF alpha, TNF beta, Fas ligand, and HUVEO91 (TNF gamma), a TNF ligand family member that Dr. Guo-Liang Yu, then a scientist at HGS, was studying (RE96 at pages 79-80 and 90-93). I discussed the August 9, 1994 BLAST result and the August 10, 1994 alignment with Ms. Ferrie within a week of August 10, 1994, and I communicated to her that it confirmed for me that the AIM-I protein encoded by HTPAN08SO4 was a full-length member of the TNF ligand family with a high degree of homology to TNFα and FasL and, like those two proteins, would be useful in inducing apoptosis in certain cells. I indicated this use for AIM-I protein in the project report created and modified on February 2, 1994 (RE97 at page 1) and in the project report created on February 2, 1994 and modified on April 14, 1995 (RE99 at page 1).

HGS PROTOCOL FOR CLONES OF HIGH INTEREST

15. Once I recognized the high degree of homology of AIM-I to TNFα and FasL, I had the idea of developing AIM-I based therapeutics, including anti-AIM-I antibodies, for example, for treatment of autoimmune diseases, or the AIM-I protein itself, for example, for treatment of cancer. I recall discussing these ideas with my colleagues, including Reiner Gentz, and also with Ms. Ferrie around the time that we identified the homology between AIM-I and TNF ligand family members mentioned above. Based on these ideas, a decision was made at HGS to pursue the development of AIM-I therapeutics as a matter of high priority in HGS's interdepartmental program. Designating AIM-I a high priority project meant that HGS committed resources to my work on AIM-I to the exclusion of hundreds of other potential therapeutic proteins also being evaluated during this time period. HGS's program for high priority projects involved a variety of discovery platforms in place at HGS to obtain as much information about AIM-I in the quickest manner possible to guide the development of AIM-I related therapeutics. Several of the discovery platforms applied to AIM-I, such as the fluorescence *in situ* hybridization to detect chromosomal location of the AIM-I gene and the indirect immunofluorescence studies of AIM-I protein, as discussed in paragraphs 21 and 30 below, were utilized virtually exclusively in development of therapeutics from molecules of high interest.

16. The initial step in developing AIM-I therapeutics was to obtain a full length clone of AIM-I. We obtained and sequenced a full length clone of AIM-I no later than August 8, 1994 (RE96 at 73-78), therefore, I must have had my ideas regarding the development of AIM-I therapeutics, and HGS must have made the decision to make the development of AIM-I therapeutics a "high interest" project, prior to this August 8, 1994 date.

17. One discovery platform used for developing AIM-I therapeutics was the protein expression platform. In addition to the bacterial expression of AIM-I performed by Ms. Ferrie (see paragraph 25), the Protein Expression Group at HGS, under the supervision of Dr. Reiner Gentz, spent extensive efforts to develop an insect expression system for AIM-I as part of a TNF ligand and TNF receptor family expression program, as described in paragraph 26 below. Additionally, expression of AIM-I in the rabbit reticulocyte lysate system was performed (paragraphs 27 and 28 below) to ensure that the proper signals for protein translation were present in our AIM-I clones. The assessment of a variety of expression constructs in a variety of expression systems allowed us to identify the most suitable scheme for expressing large amounts of AIM-I protein in the fastest and most economic manner possible for the development of AIM-I related therapeutics.

18. In addition to the protein expression platform, bacterially-produced AIM-I protein was sent to the Pocono Rabbit Farm and Laboratories for the generation of anti-AIM-I antibodies (see paragraph 31 below) in June of 1995. In that time period, antibody production was part of a protocol in place at HGS for characterizing and developing molecules of high therapeutic interest.

19. Another discovery platform in place at HGS for high interest genes was expression analysis through Northern blotting (see paragraph 29 below). Northern blotting is used to assess the tissue specificity and cell specificity of expression of the AIM-I gene. This information, for example whether or not AIM-I is expressed in cancer cells and immune system cells, is important for developing animal models and test protocols when assessing AIM-I therapeutics.

20. Yet another discovery platform in place at HGS for the development of therapeutically relevant molecules was indirect immunofluorescence, which involved expressing epitope-tagged proteins in mammalian cells to evaluate whether the proteins were properly processed and localized in the appropriate subcellular compartment. Because this platform demanded a considerable amount of HGS resources, its use was limited to molecules that were deemed to be of a very high priority for therapeutic development. AIM-I was considered to be such a molecule, and thus indirect immunofluorescence of AIM-I protein was performed by Ms. Ferrie (see paragraph 30 below).

21. In addition to the development platforms described in paragraphs 15 through 20 above, the chromosomal location of AIM-I was determined by the HGS Exploratory Research group, headed by Ken Carter (see paragraphs 34 to 37 below) as part of the AIM-I therapeutic development program. This mapping experiment was carried out to correlate the physical position of AIM-I on the chromosome with known genetic map data and to further elucidate the biological role of AIM-I and its value as therapeutic agent. In particular, such mapping data provides useful information as a genetic marker in mapping the human genome, in localizing chromosome regions associated with diseases such as cancer, and in potentially correlating AIM-I and diseases that may map to the same chromosomal region. They also confirm that the cDNA is encoded by a specific gene in the human genome, and, therefore, encodes a naturally occurring protein. The human AIM-I gene was determined to specifically map to chromosome 3q25-27. Because of the labor-intensive nature of chromosome mapping, this type of analysis was reserved solely for proteins of the highest therapeutic interest at HGS.

22. The efforts undertaken in the development of AIM-I therapeutics, as summarized in paragraphs 15 through 21 above, took place in 1993 through 1996. These efforts reflect an

immense commitment on HGS's part at a time when HGS, founded in 1992, was no more than a young start-up company.

PRODUCTION AND CHARACTERIZATION OF AIM-I PROTEIN

23. Once I had determined that a full-length cDNA encoding AIM-I had been cloned, I directed a great deal of effort from early 1994 up to and beyond March 14, 1996 to produce the full-length AIM-I protein in a variety of gene expression systems, as detailed below. The activities carried out in my laboratory in this endeavor from early 1994 up to and beyond March 14, 1996 are recorded in part in Ms. Ferrie's laboratory notebooks (HGS Notebook Numbers 82, 127, 168, 202, 236, 270, 345, 405, and 489, labeled by Ms. Ferrie as Ann Kim #3, #5, #6, #7, #8, #9, #10, #11, and #12; RE87 - RE95, respectively). Recombinant production of AIM-I protein is one of the first steps one must take in order to prepare the protein to generate what I refer to herein as "AIM-I therapeutics," such as anti-AIM-I antibodies for therapeutic use, for example, for treatment of autoimmune diseases, or therapeutics comprising the protein itself, for example for treatment of cancer. Also, protein production *in vitro* and *in vivo* is generally helpful for determining the various characteristics of a new protein, such as its membrane topology, glycosylation pattern, and posttranslational processing, if any, and the correct (*i.e.* natural) protein translation initiation site. In addition, I directed the production of recombinant AIM-I as a reagent for analyzing its biological activity, and for raising anti-AIM-I antibodies for analyzing the expression patterns and function of the protein and for developing the antibodies as therapeutic reagents.

24. In January 1995, I directed the successful bacterial expression and subsequent purification of AIM-I protein. Expression constructs encoding two different forms of AIM-I were generated in order to develop an expression system that yielded high levels of AIM-I

protein to facilitate development of AIM-I therapeutics: (a) construct HTPAN08SO4 185bp was used to generate a protein with translation beginning at the 45th residue of the AIM-I open reading frame, *i.e.* methionine 45 ("Met-45"); and (b) construct HTPAN08SO4 51 bp was used to generate a protein with translation beginning at the first residue of the open reading frame, *i.e.* methionine 1 ("Met-1"). These two forms of AIM-I were cloned into three different vectors, pD10, pQE60 and pA2. pD10 and pQE60 are bacterial expression vectors whereas pA2 is a baculovirus vector for expression in Sf9 insect cells.

25. Bacterial expression of AIM-I provided an abundant source of AIM-I antigen used for the production of polyclonal antibodies. Moreover, AIM-I expressed in bacteria provided a potential source of protein for use in assays designed to demonstrate apoptotic activity.

26. Insect cell expression provides for the production of protein having a eukaryotic glycosylation pattern. The Sf9 system is one of three major, routine expression systems that have been used at HGS to determine the optimal expression system. Once Ms. Ferrie made the AIM-I Sf9 expression constructs, the constructs were provided to the HGS Protein Expression Group, headed by Dr. Reiner Gentz, for expression of AIM-I in Sf9 insect cells. I communicated with Dr. Gentz regarding the progress of this project on a regular basis, and Dr. Gentz kept me abreast of the significant efforts in 1995 to express AIM-I in the insect cell system. In addition to expression of AIM-I protein, the Protein Expression Group was evaluating expression systems for the TNF ligand family members TNF gamma (also referred to as VEGI or HUVEO91) and TNF delta. Because of the high degree of similarity among TNF ligand family members, we anticipated that information regarding suitable expression systems for TNF ligands such as TNF gamma and TNF delta would also be useful to determine the most

suitable approach to express AIM-I protein for the development of AIM-I therapeutics. Among the members of the Protein Expression Group working on elucidating suitable expression conditions of AIM-I and other TNF ligand family members in 1995 and 1996 under the ultimate supervision of Dr. Gentz were Dr. Timothy Coleman, Dr. Guo-Liang Yu, Solange Gentz (then known as Solange Lima), Lily Xing, and Markus Buergin.

27. Rabbit reticulocyte lysates are commonly used for *in vitro* translation of messenger RNA transcripts. Many commercial kits are available for the performance of this assay, wherein the user provides the mRNA encoding the protein of interest. This experimental system can be used to answer a number of important questions about a protein and its encoding nucleic acids. For example, it is convenient for determining whether a correct cDNA construct has been assembled and isolated. If complementary RNA (cRNA) transcribed from a cDNA construct under study can be translated *in vitro* to produce a protein in reticulocyte lysates, then the user is assured that the construction encodes viable translation initiation and termination signals, *i.e.*, it confirms that the gene is expressible.

28. In vitro expression of AIM-I protein was achieved by August 25, 1994 (RE90 pages 33-34). The significance of AIM-I expression was at least fourfold: (a) confirmation of the correctness of a properly transcribable and translatable reading frame; (b) confirmation of the correctness of the predicted molecular weight; (c) production of a reagent suitable for use in antibody production; and (d) production of a reagent for demonstration of therapeutic use in biological activity assays (*e.g.* killing cancer or immune cells).

29. Northern blots, or RNA blots, provide an indication of the tissue specificity and cell specificity of expression of AIM-I. The mRNA expression pattern is useful in predicting, confirming, or even determining the function of a new gene, such as AIM-I. In particular,

determining the expression pattern of AIM-I would be useful in identifying the likely target receptor and target cell type for AIM-I. Northern analyses were carried out by Ms. Ferrie under my direction in December 1995 for the purpose of determining the expression pattern and tissue specificity of AIM-I.

30. Indirect immunofluorescence analysis of the AIM-I protein was undertaken as part of my overall effort to obtain a detailed understanding of the biological properties of AIM-I as they exist in a living mammalian cell, and to ensure proper processing of the AIM-I protein to produce a biologically active protein. Generally, immunocytochemistry can be used to reveal the subcellular localization of a protein. Moreover, a protein under study can be genetically engineered to display antigen tags at various locations along the linear sequence of the protein, such as at the N-terminus and the C-terminus. The tagged protein can then be subjected to immunocytochemical analysis to reveal the subcellular localization of specific antigen-tagged portions of the protein. Such an approach can reveal important information about protein processing, topology, and orientation within cellular membranes. The immunofluorescence experiments that Ms. Ferrie carried out under my direction in 1995 were designed to determine the sub-cellular location of the AIM-I protein.

31. As noted above, bacterially produced recombinant AIM-I was used as a source for raising anti-AIM-I antibodies. These anti-AIM-I antibodies were raised by Pocono Rabbit Farm and Laboratories (PRF&L) in rabbits using recombinant AIM-I protein that Ms. Ferrie produced and sent to PRF&L under my direction in June of 1995; and the antibodies produced were sent to my laboratory by PRF&L throughout the production process until the final exsanguination of the rabbits and receipt of the final antisera at HGS in January 1996. These antibodies, samples of which were used by Ms. Ferrie under my direction in Western analyses between August 30, 1995

and March 14, 1996, were raised for the purpose of studying the expression patterns and activity of the AIM-I protein, as well as confirming its production through recombinant constructs.

JOINT PROGRAM WITH SMITHKLINE BEECHAM

32. Because of the great interest within HGS in the TNF ligand family, including in particular AIM-I, a joint program was established between HGS and SmithKline Beecham ("SB") (the "HGS/SB Joint Program") to commit greater resources toward developing these ligands and their receptors as potential therapeutic agents. Dr. Reiner L. Gentz, who at the time was Director of Protein Expression and Purification at HGS, managed HGS's involvement in this HGS/SB Joint Program. Thus, in addition to the work being conducted on AIM-I under my direction in my laboratory and in other HGS departments as described herein, the HGS/SB Joint Program included activities by Dr. Jian Ni, Dr. Guo-Liang Yu, Dr. Yu's assistant Lily Xing, each of HGS; and Alem Truneh and several scientists at SB. From time to time during the HGS/SB Joint Program, including during the 1995 to 1996 time period, members of the HGS/SB Joint Program held official meetings to discuss their progress in this program. One such meeting took place on October 18, 1995, and although I did not attend that meeting, I reviewed the meeting abstracts and minutes (RE71) shortly after the meeting. In addition to obtaining information from formal meetings of the HGS/SB Joint Program, I communicated directly with HGS and SB personnel about the progress of analysis of the TNF Ligand and Receptor family members, including AIM-I.

33. To better understand the role and mechanism of AIM-I apoptotic activity, it was important to identify such TNF receptor family members to ultimately determine which receptors did and did not bind AIM-I. Also key to this analysis of AIM-I function was to compare and contrast the expression patterns of the various members of both the TNF ligand (e.g. TNF

gamma, delta, and epsilon) and TNF receptor (e.g. TR1, TR2, and TR3) families, and thereby identify the likely target receptors and target cell lines for AIM-I. Therefore, work carried out by HGS and SB scientists from early 1995 through March 1996 on various TNF ligand and receptor family members, such as TNF gamma, delta, and epsilon, and TRI, TR2, and TR3 all contributed to further elucidating the biological function of AIM-I.

CHROMOSOMAL MAPPING

34. In late 1995, I undertook to map the chromosomal locus of the AIM-I gene. This mapping experiment was carried out to correlate the physical position of AIM-I on the chromosome with known genetic map data and to further elucidate the biological role of AIM-I and its value as therapeutic or diagnostic agent. In particular, such mapping data provides useful information as a genetic marker in mapping the human genome, in localizing chromosome regions associated with diseases such as cancer, and in potentially correlating AIM-I and diseases that may map to the same chromosomal region. They also confirm that the cDNA is encoded by a specific gene in the human genome, and, therefore, encodes a naturally occurring protein. The human AIM-I gene was determined to specifically map to chromosome 3q25-27.

35. On November 29, 1995, I made an internal HGS request for chromosomal mapping of the AIM-I gene, as indicated on the standard HGS order form DNA Probe for Chromosomal Mapping Request #279 order form, dated November 29, 1995 RE103. I made this request for the intended purpose of mapping the AIM-I gene and I expected the clone to map to a specific position in the human genome.

36. On November 30, 1995, an AIM-I nucleic acid probe was labeled for the chromosomal mapping procedure by Meena Augustus, who was a chromosomal mapping

technician in the HGS Exploratory Research department supervised by Ken Carter at that time (Augustus notebook 346 RE104 at page 97).

37. On December 5, 1995, AIM-I was determined to map to chromosome 3q25-27; and on December 6, 1995, the mapping information was entered into IRIS RE104 at page 101). The result of the mapping experiment was communicated to me by either Ken Carter or one of his assistants within two to three days of December 5, 1995.

PATENT RELATED ACTIVITY

38. I personally assisted in the preparation of a provisional patent application directed to AIM-I. On or about January 30, 1996, I completed and forwarded to the HGS Legal Department a patent questionnaire to be used in preparing the provisional patent application RE105

39. On or about February 12, 1996, I delivered samples of AIM-I cDNA (DNA Plasmid 413412) encoding AIM-I that Ms. Ferrie had aliquoted out to the HGS Legal Department for depositing with the American Type Culture Collection in connection with filing a provisional patent application directed to AIM-I (RE95 page 20).

40. On March 8, 1996, I discussed the preparation of the provisional patent application directed to AIM-I by telephone with Charles Herron, outside patent counsel at Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein ("Carella") (RE50).

41. On March 14, 1996, I directly corresponded by telefacsimile with Mr. Herron of Carella regarding the final preparation of the aforementioned provisional application, including providing Mr. Herron with data and materials to be included in the application (RE51).

42. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or any patent issuing thereupon.

Date: 6/24/04

Steven M. Ruben

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